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Alexander Clark

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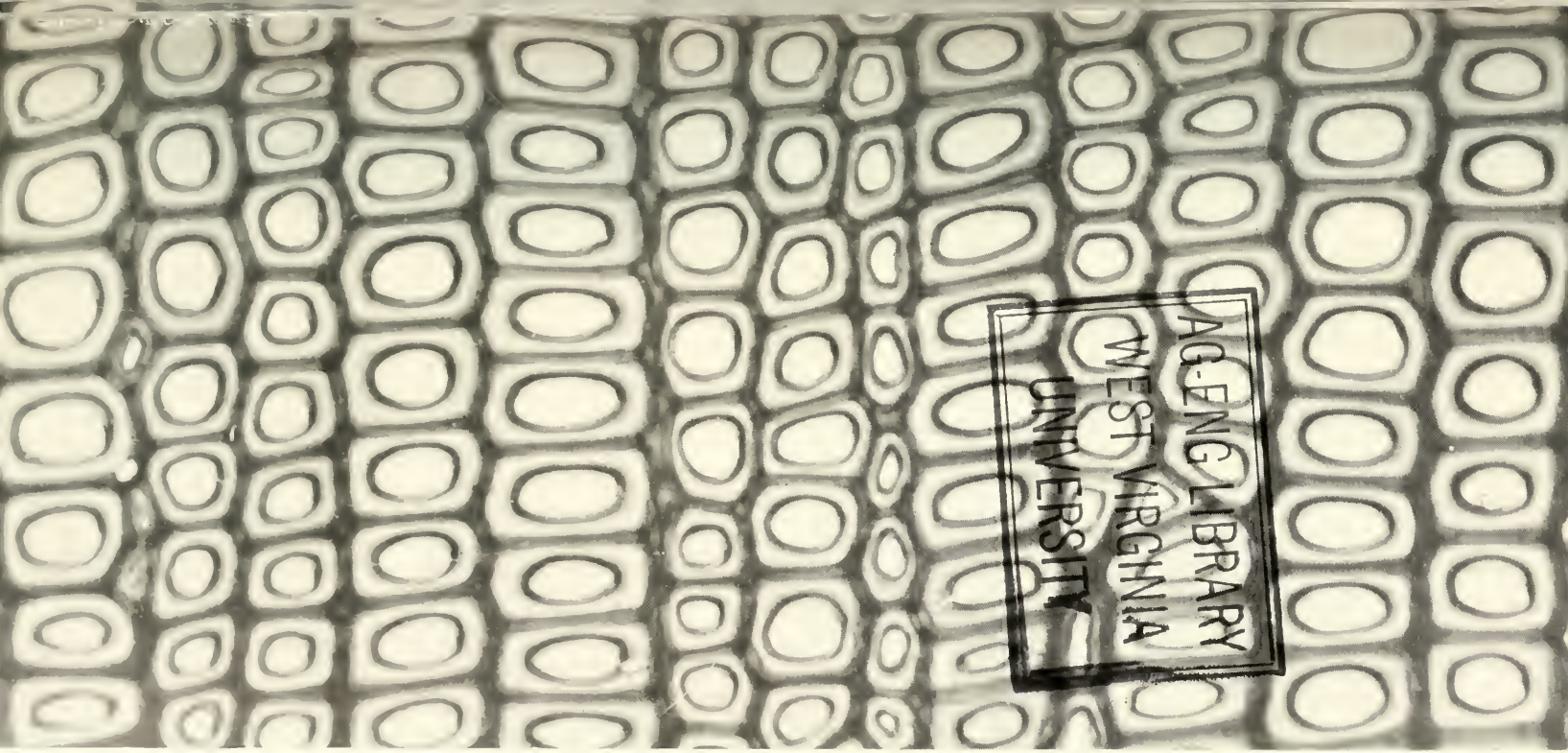

Effects of Ionizing Irradiation on the Xylem Derivatives of Pinus Echinata Mill.

West Virginia University Agricultural Experiment Station

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THE AUTHORS

Alexander Clark III is Wood Technologist, Southeastern Forest Experiment Station, U. S. Forest Service. J. R. Hamilton is Wood Scientist, West Virginia Agricultural Experiment Station.

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West Virginia University
Agricultural Experiment Station
College of Agriculture and Forestry
A. H. VanLandingham, Director
Morgantown

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marked so that the direction of the reactor could be ascertained later. Two longitudinal strips, measuring one inch in the tangential direction and of sufficient length in the radial direction to include the 1958 through 1963 increments, were removed from each bolt, one on the side toward the reactor and the other on the side diametrically opposite. In this way, each tree was sampled at ten locations—five height levels and two sides at each height level.

Within each sample the specific tissues of interest were those formed during radiation exposure in 1959 and 1960 and comparable tissues in the 1958 increment. Because usable control trees would have of necessity been located at some distance from the irradiated trees, the 1958 increment in each tree was used for control purposes.

Three longitudinal sections 100 microns in thickness (radial direction), were cut on a sliding microtome from the selected annual increments. Care was exercised, by frequent comparisons between transverse sections prepared for this purpose and the block being cut, to insure that the longitudinal sections cut from both the irradiated tissues in June, 1959 and in August, 1960 and from the 1958 controls occupied equivalent within-increment positions. These sections were catalogued and stored in distilled water for a maximum of three days.

Tensile test specimens were punched from the wet sections with a specially constructed die to final size of 0.12 x 3.35 inches. Care was taken to eliminate cross grain. The saturated specimens were stressed over a 1.52 inch length at an elongation rate of 0.005 inches per minute using an Instron floor model testing machine. Pneumatic action grips with line bar grip faces were used to eliminate slippage during the test. The tensile load at failure was recorded and converted to pounds per square inch (psi) for each specimen. The average of the results of the three tests was used as a measure of the tensile strength in each increment and location sampled.

After testing, each micro-tensile test specimen was macerated in equal parts of glacial acetic acid and hydrogen peroxide. Measurements were made of wall thickness, lumen diameter and length of ten randomly selected, isolated, vertical tracheids using a projection microscope and calibrated rule. Total lengths and the diameter and wall thickness midway of the cell were recorded. Data from the three specimens were averaged and used as a measure of the tracheid characteristics for each increment in each location.

In analyzing the data two separate analyses of variance were executed. The first, a preliminary analysis made after data from two trees had been collected, employed a split-plot design and was used to determine whether the timing of irradiation with respect to growth period produced differ-

ential effects and whether there was a difference between sides. The differences due to trees, growth periods, and sides constituted the whole-plot while the difference due to height were sub-plots. Each of the characteristics was analyzed separately.

Based on results of the preliminary analysis, the remaining two trees were sampled only on the side nearest the source. The second analysis of variance also made use of a split-plot design and was used to determine if there was a significant degree of variation among the four exposed trees using only the data from the side closest to the source. Effects of irradiation and effects of trees constituted the whole-plot and the differences due to height were sub-plots.

After the first analysis it was noted that the error for the whole-plot was consistently smaller than the error for the sub-plot. Since this can occur only by chance, both error terms were considered to be estimates of the same population variance. Consequently the sums of squares were pooled in order to obtain a more accurate estimate of the population variance (6). The irradiated tissues produced in June, 1959, and in August, 1960, were analyzed separately for each variable considered.

Results and Discussion

The effects of ionizing radiation on shortleaf pine tissues may be noted in Figure 1 as zones of irregularly shaped cells occurring in the latewood of the 1959 and 1960 increments. Tracheids differentiated during irradiation appear to have larger lumina, thinner walls, and a distorted shape in contrast to those produced under normal conditions. As reported by several investigators (1, 2, 3), the cell abnormalities associated with irradiation have distinct boundaries. Rapid changes occur both with inception and cessation of irradiation.

Irradiation during August, 1960 caused the formation of anomalous tissues in the latter portion of the latewood at each height level. In contrast, the location of aberrations associated with the June, 1959 irradiation was not constant within the increment but varied with height. From the lowest point sampled to approximately 50 per cent of total height the anomalous tissues were located in the latewood; in the upper bole, they were observed in the latter portion of the earlywood (Figure 2). This dependency on height was noted on both sides of all trees examined. It is evident that the June burst of irradiation occurred at the time the study trees were undergoing the transition from earlywood to latewood tissue production since latewood initiation has been noted to begin in early June and progress acropetally at a gradual rate (9). It has been postulated that the change in cell character within increments reflects changes

in auxin concentration associated with changes in the rate of shoot elongation (4). Results obtained here suggest that irradiation did not disturb the normal pattern of auxin synthesis or transport.

Because of the difference in within-increment location of irradiated

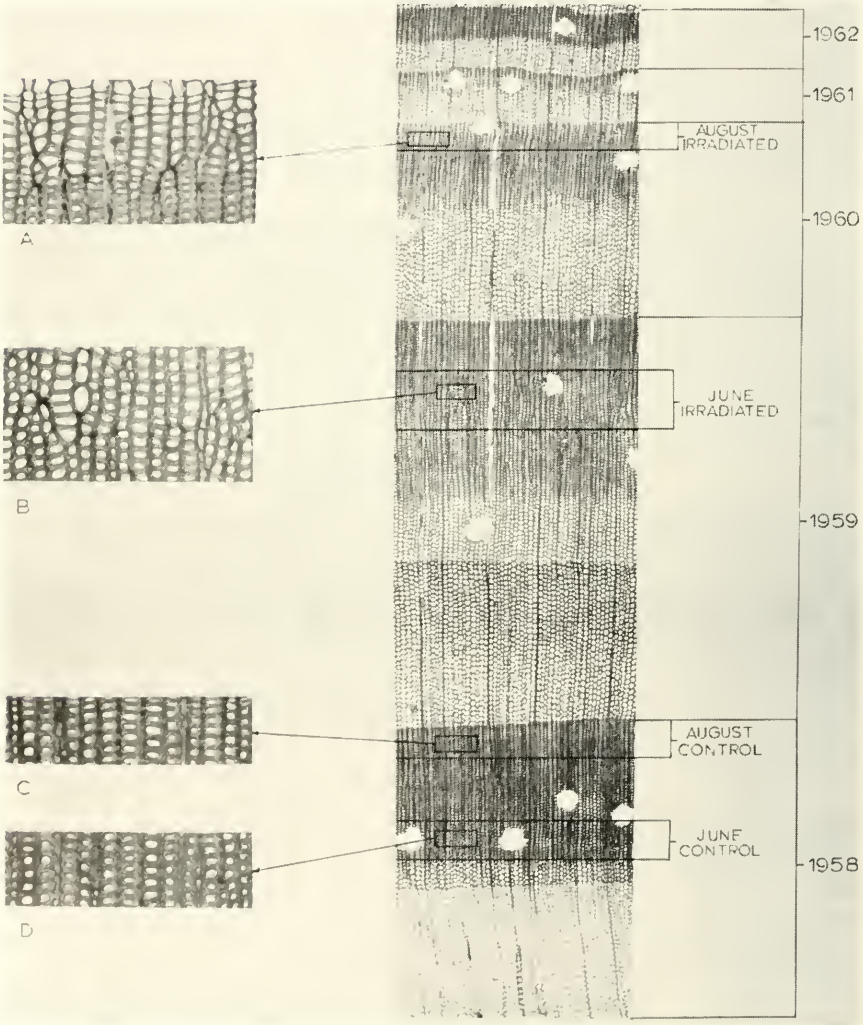

Figure 1. The location of sampling areas at the lowest height level in shortleaf pine. A and C are details of the paired areas representing the August 1960 irradiation period and its 1958 control. B and D are details of the paired areas representing the June 1959 irradiation period and its 1958 control. (Composite photomicrograph x 50, detail x 190)

tissues associated with the June period of irradiation, the test specimens were located in the latewood in the lower half of the trees and in the earlywood in the upper half of the trees. All of the specimens representing the August, 1960 irradiation period were located in the latewood.

In the preliminary analysis, orthogonal comparisons revealed that there was a difference between irradiated and control tissues, and that the effects in June were not the same as those in August when wall thickness, lumen diameter, and tensile strength were considered (Table 1). In addition, there was no significant difference between the side nearest and the side opposite the reactor which could be attributed to irradiation. Based on these results, the subsequent analysis treated the 1959 and 1960 irradiation periods as distinct events. They were analyzed separately, and the properties were determined only on the side of the trees toward the source.

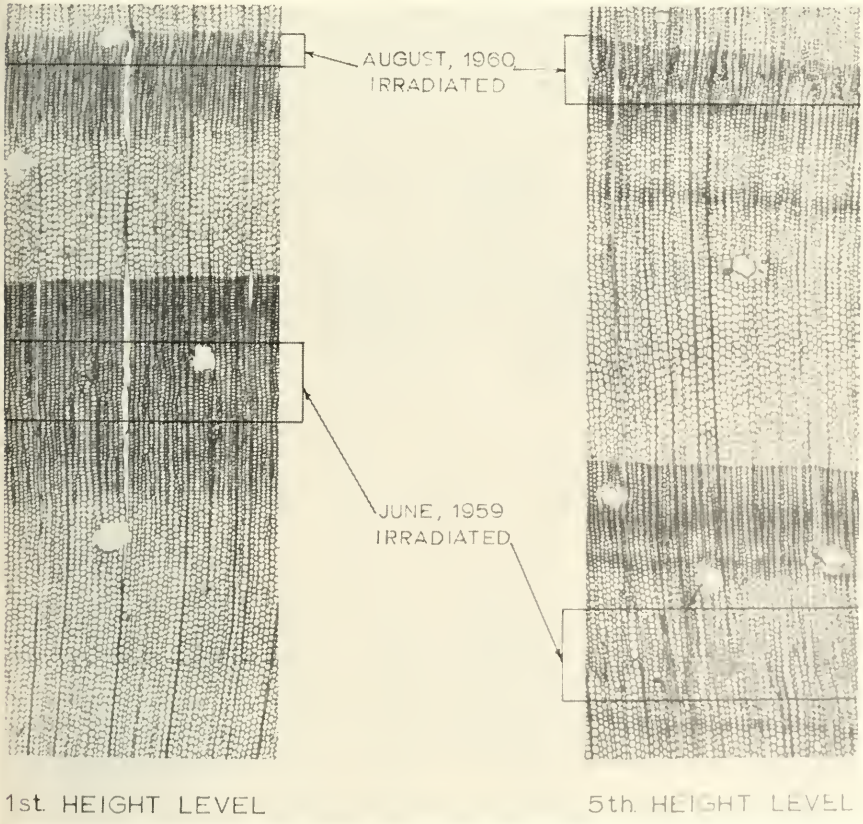

Figure 2. The xylem of shortleaf pine illustrating the effect of height on intra-increment location of radiation induced abnormalities.

TABLE 1

Analysis of variance for four characteristics of shortleaf pine tissues formed during irradiation in June, 1959 and August, 1960. The analysis incorporates data from two sides at five height levels in two trees.

Source	df	Mean Squares: Tensile Strength	Cell Wall Thickness	Lumen Diameter	Tracheid Length
		psi	*u*	*u*	*mm*
Trees	1	25611502.75*	1.3363	108.472**	0.0173
Growth periods	3	222029754.00**	77.525**	1052.550**	0.038
Irradiated vs. control	1	149180875.20**	16.983*	51.520*	0.062
June vs. August	1	439654710.60**	203.075**	3023.340**	0.029
Interaction	1	77253977.80**	12.466*	82.662**	0.021
Sides	1	21198434.50	0.319	11.953	0.019
Sides X growth periods	3	14338798.13	0.974	4.538	0.007
Error a	7	4220432.87	2.089	4.555	0.144
Height levels	4	118833492.00	66.443**	317.884**	3.060**
Growth periods X height levels	12	11771591.13	5.101**	86.609**	0.103
Sides X height levels	4	5396011.50	4.389	26.561*	0.406*
Growth periods X sides X height levels	12	6339473.56	0.374	12.737	0.051
Error b	32	6045417.56	0.613	6.973	0.109

* Significant at the .05 level of probability
** Significant at the .01 level of probability

Tracheid wall thickness and lumen diameter were significantly altered by the August, 1960 irradiation (Table 2) but not by June, 1959 irradiation (Table 3). Tracheid length was not affected in either year. When all sample locations in the four trees were considered collectively, a 16 per cent reduction in all thickness and a 31 per cent increase in lumen diameter were noted in the 1960 increment. Similar results have been reported in *Pinus rigida* and *P. echinata* (3), but, in these studies, significant reductions in length were also noted. In the present study only small and non-significant reductions in length were recorded. Lack of agreement may stem from inadequate sample size in the present study.

In both the preliminary analysis and the analyses of four trees it was shown that there was a difference in the effect of irradiation in June, 1959 and August, 1960. These differences may have resulted from three contributing factors. The 1960 irradiation was 55 per cent greater than that of 1959, a factor which in itself may account for the differential response. It should be remembered, however, that the 1960 exposure was in addition to that which had been accumulated during 1959. The possibility also remains that there is a difference in radiosensitivity between differentiation of earlywood and latewood tissues and in the processes which govern their formation. The changes which occurred in the latewood resulted in cells which had characteristics resembling earlywood cells—thinner walls and larger lumina.

Strength in tension parallel to the grain was also affected by irradiation (Table 4). As with cell characteristics, significant effects were noted in the 1960 increment only (Tables 2 and 3), where maximum average tensile strength was 11,454 psi or 26 per cent less than that of the control.

In each analysis a significant tree-to-tree difference was indicated. On the basis of an examination of the per cent change between irradiated and control specimens for each tree, the differences appear to be a reflection of inherent tree differences rather than differences in radiation response.

All four of the characteristics measured varied significantly with height in both irradiation periods (Table 2 and 3). The changes with height in the 1959 irradiation period (Figures 3 and 4) reflect not only changes associated with height *per se* but also the change in within-increment location of the irradiated tissues. A comparison of the slopes of the curves for irradiated and control specimens in Figures 3 and 4 suggests that the significant height effect was actually due to the normal variation pattern in coniferous species. Statistical evidence that the radiation effects were not a function of height occurs in the lack of significance of the radiation versus control and height level interaction (Tables 2 and 3).

TABLE 2

Analysis of variance for four characteristics of shortleaf pine tissues, irradiated in August, 1960, from the side toward the reactor in four trees.

Source	df	Mean Squares			
		Tensile Strength	Cell Wall Thickness	Lumen Diameter	Tracheid Length
		psi	*u*	*u*	*mm*
Trees	3	23602712.25**	6.49**	37.185**	0.164
Irradiated vs. control	1	167146056.00**	25.729**	160.322**	0.125
Height levels	4	43267164.50**	16.679**	32.601**	1.209**
Trees X height levels	12	9166459.75*	0.707	4.055	0.138
Irradiated vs control X height levels	4	2978039.09	0.293	4.499	0.145
Among first 4 levels	3	3318549.46	0.325	1.844	NS
First 4 vs. 5th	1	1956508.00	0.199	12.466	NS
Error	15	1886030.72	0.341	3.991	0.057
Total	39	561222984.00	126.471	528.807	8.548

* Significant at the .05 level of probability
** Significant at the .01 level of probability

TABLE 3

Analysis of variance for four characteristics of shortleaf pine tissues, irradiated in June, 1959, from the side toward the reactor in four trees.

Source	df	Mean Squares			
		Tensile Strength	Cell Wall Thickness	Lumen Diameter	Tracheid Length
		psi	*u*	*u*	*mm*
Trees	3	15379510.00**	5.368**	87.666**	0.469**
Irradiated vs. control	1	2276244.09	0.111	0.497	0.107
Height levels	4	62598834.00**	38.092**	373.885**	1.797**
Trees X height levels	12	12806634.00**	2.759*	32.939*	0.103
Irradiated vs. control X height levels	4	1973879.03	0.581	5.174	0.177
Error	15	1747762.91	0.663	9.015	0.047
Total (SS)	39	486601676.00	213.966	2310.223	11.357

* Significant at the .05 level of probability
** Significant at the .01 level of probability

TABLE 4

Means for four characteristics of shortleaf pine tissues formed during irradiation and in control tissues.

Item	Tensile Strength	Cell Wall Thickness	Lumen Diameter	Tracheid Length
	psi	*u*	*u*	*mm*
		1959		
Control	8063.2	6.0	27.8	3.2
Irradiated	7586.0	6.1	28.0	3.1
Difference	477.2	0.1	0.2	0.1
Per cent change	5.9	1.8	0.8	3.3
		1960		
Control	15542.6	10.2	13.1	3.3
Irradiated	11454.2	8.6	17.1	3.1
Difference	4088.4	1.6	4.0	0.1
Per cent change	−26.3	−15.7	+30.6	−6.1

Further statistical examination by means of orthogonal comparisons (Table 2) also confirms the uniformity in the distribution of radiation effects in the tree boles. This conclusion is not in agreement with the reports of others (8) who suggest that radiation effects are not uniformly distributed throughout the boles of trees but are conditioned by proximity to the living crown.

In addition to the cell aberrations which were evident from the quantitative measurements, other abnormalities were frequently encountered. All irradiated tissues were composed of cells which were very irregular in cross sectional outline, reflecting unusual lateral expansion which appeared to be occasioned by an increased frequency of cambial additions and deletions. Such abnormalities have been related to an enlargement of medullary ray cells in *Pinus monophylla* (1). When individual cells were observed in longitudinal section, other curiosities were apparent. The frequency of bifurcations was increased greatly over that which is usually observed in shortleaf pine. They were more extensive, at times involving one-half of the cell length (Figure 5A), and were considerably more irregular (Figure 5B). Many tracheids were penetrated by holes of varying size which showed some evidence of secondary wall development (Figure 5C) resulting in a cylindrical formation. These structures extended for some distance in contiguous cells in the radial direction. Formations similar to these known as trabeculae occur frequently in *Chamaecyparis nootkatensis* and to a limited extent in *Pinus monticola* (5). The formation of trabeculae has been related to the penetration of fungal hyphae in the cambium.

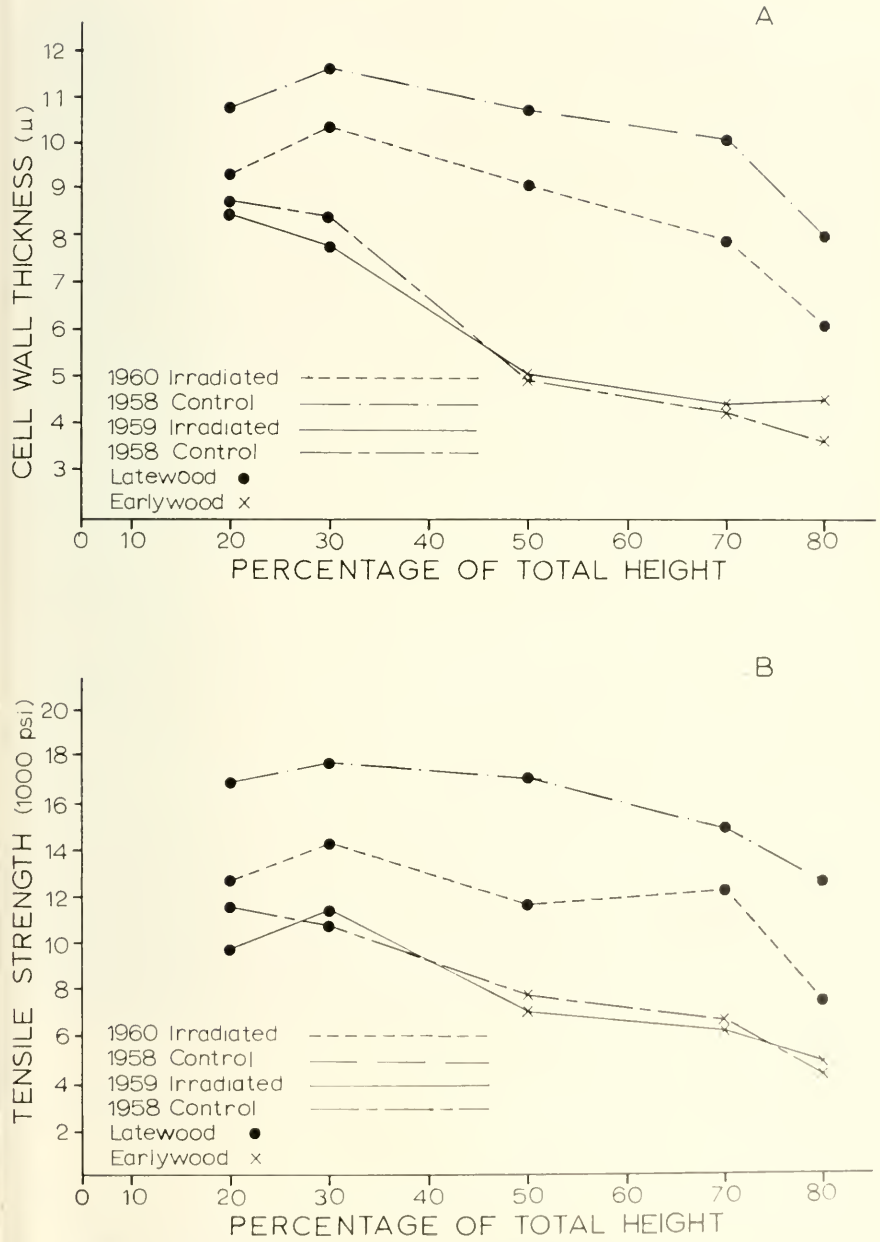

Figure 3. The variation of cell wall thickness (A) and longitudinal tensile strength (B) with height in control and irradiated shortleaf pine.

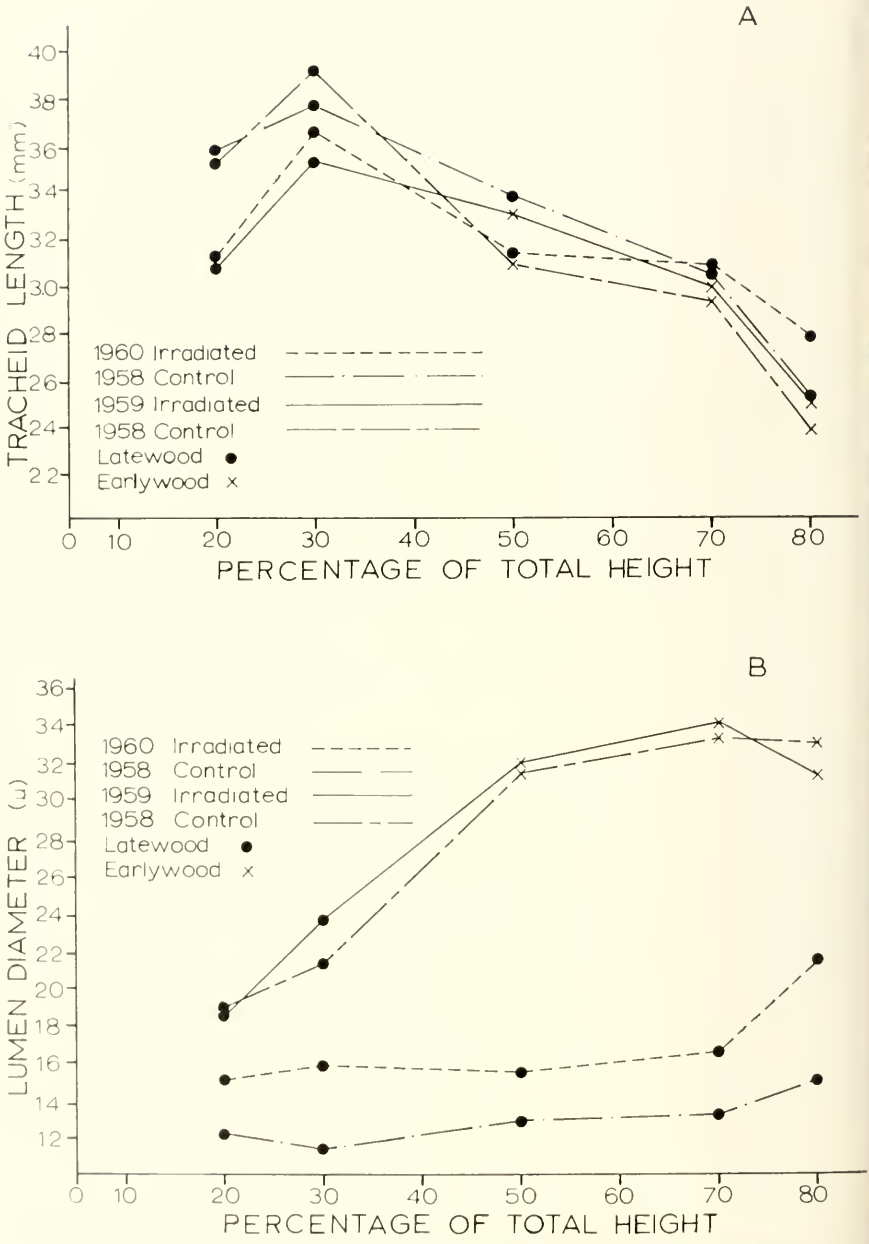

Figure 4. The variation of tracheid length (A) and lumen diameter (B) with height in control and irradiated shortleaf pine.

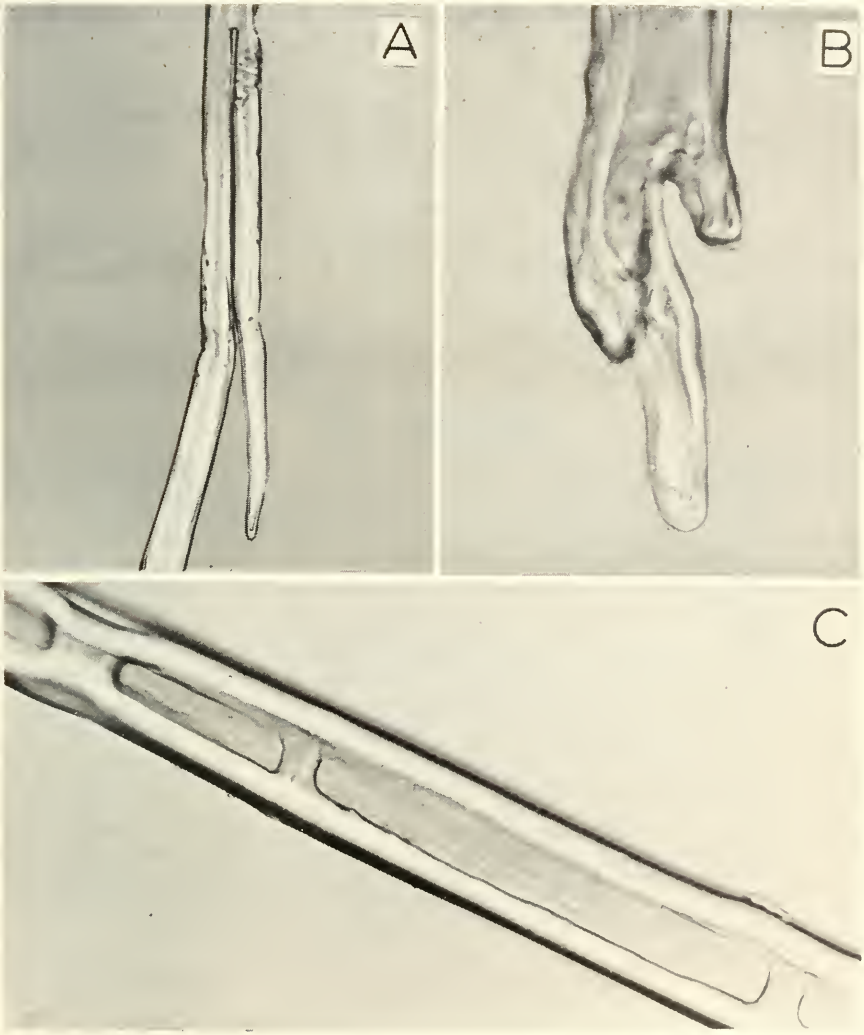

Figure 5. Tracheid abnormalities in irradiated shortleaf pine: A and B extensive and irregular bifurcations (x 800) ; *C abnormal secondary wall formations* (x 1200) .

Conclusions

The data presented in this bulletin appear to justify the following conclusions:

1. At the levels of ionizing radiation studied, the secondary xylem of shortleaf pine is subject to radiation induced changes. The

changes are uniformly distributed in the tree boles; no effect of height or of orientation with respect to the direction of the source were noted. The observed changes appear to be superimposed on the normal patterns of variation which characterize this species.

2. Initial exposure of 900 rads was not sufficient to cause changes in wall thickness, lumen diameter or length of vertical tracheids. However, exposure the following year to 1,400 rads caused measurable changes in two cell characteristics and tensile strength.
3. Following exposure to accumulated exposures as great as 2,300 rads a relatively rapid return to normal cell production and differentiation occurs.

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